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Characterization of G-protein α subunits in the G_q class: Expression in murine tissues and in stromal and hematopoietic cell lines

(heterotrimeric GTP-binding protein/signal transduction/multigene family/phospholipase C)

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ABSTRACT Murine $G\alpha 14$ and $G\alpha 15$ cDNAs encode distinct α subunits of heterotrimeric guanine nucleotide-binding proteins (G proteins). These α subunits are related to members of the G_q class and share certain sequence characteristics with $G\alpha_q$, $G\alpha 11$, and $G\alpha 16$, such as the absence of a pertussis toxin ADP-ribosylation site. $G\alpha 11$ and $G\alpha_q$ are ubiquitously expressed among murine tissues but $G\alpha 14$ is predominantly expressed in spleen, lung, kidney, and testis whereas $G\alpha 15$ is primarily restricted to hematopoietic lineages. Among hematopoietic cell lines, $G\alpha 11$ mRNA is found in all cell lines tested, $G\alpha_q$ is expressed widely but is not found in most T-cell lines, $G\alpha 15$ is predominantly expressed in myeloid and B-cell lineages, and $G\alpha 14$ is expressed in bone marrow adherent (stromal) cells, certain early myeloid cells, and progenitor B cells. Polyclonal antisera produced from synthetic peptides that correspond to two regions of $G\alpha 15$ react with a protein of 42 kDa expressed in B-cell membranes and in *Escherichia coli* transformed with $G\alpha 15$ cDNA. The expression patterns that were observed in mouse tissues and cell lines indicate that each of the α subunits in the G_q class may be involved in pertussis toxin-insensitive signal-transduction pathways that are fundamental to hematopoietic cell differentiation and function.

G proteins mediate signal transduction between ligand-bound seven-transmembrane-segment receptors and intracellular effectors, such as phospholipases, adenylyl cyclase, and ion channels (1–3). G proteins are heterotrimeric, composed of α , β , and γ subunits. The α subunit binds guanine nucleotide and is believed to confer receptor and effector specificity. The β and γ subunits are also required for receptor interaction and can regulate effector function (4). To explore the diversity of G-protein-mediated signal-transduction pathways, we developed an approach utilizing the PCR (5) to identify novel cDNA clones that share certain highly conserved amino acid sequences common to all G-protein α subunits. We identified cDNA clones corresponding to 8 previously uncharacterized α subunits (6–9) among a total of 16 distinct proteins expressed in mammals. Additional screens revealed several α subunits from *Drosophila* (7), *Dictyostelium* (10), *Neurospora* (K. Borkovitch, G. Turner and M.I.S., unpublished observation), and *Arabidopsis* (11). Sequence comparison of all known G proteins indicated that at least four classes of related α subunits were expressed in mammals, termed G_s , G_i , G_{12} , and G_q (3), that were also found in other animal phyla. These classifications appear to have functional relevance with respect to effector specificity; for example, α subunits of the G_s class, such as *Drosophila* and rat $G\alpha_s$ and rat $G\alpha_{olf}$, activate adenylyl cyclase (9, 12), whereas most α

subunits of the G_i class are substrates for ADP-ribosylation by pertussis toxin (PTX) and members of the G_q class were found to activate phospholipase C (PLC) β (13, 14).

In this paper we present the cDNA sequence[§] of two murine α subunits, $G\alpha 14$ and $G\alpha 15$, that are members of the G_q class of G proteins (7). The G_q class of α subunits probably regulates an array of PTX-insensitive signal-transduction pathways (15). The two previously reported members of this class, $G\alpha_q$ and $G\alpha 11$, are expressed in all murine tissues, whereas $G\alpha 14$ expression is more restricted and $G\alpha 15$ is primarily expressed in hematopoietic cell types.

MATERIALS AND METHODS

PCR. PCR amplification (16) of randomly primed cDNA with the degenerate oligonucleotides oMP19, -20, -21, and -41 (5) was done in a Perkin-Elmer/Cetus thermal cycler (denatured at 94°C for 1 min, annealed at 40°C for 1 min 30 sec, and extended at 72°C for 2 min; 35 cycles). PCR with gene-specific oligonucleotides was done in an Ericomp thermal cycler cooled by water circulated through an ice/water bath [denatured at 93°C for 10 sec, annealed at 65°C ($G\alpha 15$) or 55°C ($G\alpha 11$, $G\alpha_q$, and $G\alpha 14$) for 15 sec, and extended at 72°C for 40 sec; 25 cycles]. The gene-specific oligonucleotides used were CT25 (5'-GGGGTAGGTGATGATTGTGCG-3'), CT106 (5'-CTCGCTTAGTGCCACC-3'), CT107 (Fig. 1A), CT116 (Fig. 1B), CT117 (Fig. 1B), CT118 (Fig. 1B), CT121 (Fig. 1B), CT133 (Fig. 1B), CT144 (5'-GTGGGCCGCTAGGCACCA-3'), CT145 (5'-CTGAAGTACCCCATGTAACAT-3'), CT146 (5'-TGGCCTTAGGGTGCAGGG-3'), CT165 (5'-ATCTTCACGGCCATGCAGGCATG-3'), CT167 (Fig. 1A), CT168 (Fig. 1A), GQ4 (5'-ATTCGCTAAGCGCTACTAGA-3'), GQR (5'-AGTGGGGACACAACTCTAAGCA-3'), and oMP38 (5'-GTAGCCGACCCTTCCTATCT-3'). Each oligonucleotide was used in the PCR at 10 ng/ μ l with 100 ng of randomly primed cDNA in a 10- μ l reaction volume. PCR buffer contained 1.5 mM MgCl₂, 40 mM NaCl, 10 mM Tris (pH 8.8), and 0.01% gelatin. *Thermus aquaticus* (Taq) DNA polymerase was supplied by Cetus.

RNA Expression Analysis by PCR. Randomly primed cDNA prepared from total RNA of various tissues and cell lines was amplified by PCR using the degenerate oligonucleotides oMP20 and oMP21 combined with either oMP19 or oMP41. In separate reactions, gene-specific oligonucleotides were used to assay $G\alpha 11$ (CT165 and CT25), $G\alpha_q$ (oMP38 and GQ4), $G\alpha 14$ (CT167 and CT168), or $G\alpha 15$ (CT133 and

Abbreviations: PTX, pertussis toxin; PLC, phospholipase C; ES cells, embryonic stem cells.

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§The sequences reported in this paper have been deposited in the GenBank data base (accession nos. M80631 and M80632).

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CT116). Conditions of the PCR were held within the range of quantitative amplification to provide a relative measure of RNA content in the various samples. β -Actin primers (CT144 and CT146, 0.2 ng each) were included to provide an internal control of the PCR and did not affect amplification of the α cDNAs. The PCR products were blotted to GeneScreen (DuPont) and hybridized with radiolabeled oligonucleotides (17) specific to α_{11} (CT25), α_q (GQR), α_{14} (CT107), or α_{15} (CT117) in 5 \times Denhardt's solution/0.75 M NaCl/50 mM sodium phosphate/5 mM EDTA/0.5% SDS at 42°C for \geq 4 hr. Blots were washed as described (7). Films were exposed for several hours with intensifying screens at -70°C.

Northern and Western Analysis. Northern blots were hybridized with radiolabeled probes for α_{14} [nucleotides (nt) 1317-poly(A) (Fig. 3A)] or α_{15} [nt 68-600 (Fig. 3B) or nt 1098-poly(A) (data not shown)], washed three times (30 min each) in 5 mM Tris, pH 7.4/0.1 mM EDTA/0.5% SDS at 60°C. Western blots were prepared with reagents and procedures as described (18).

Cell Culture. All B-cell lines were maintained in RPMI 1640 supplemented with 10% fetal bovine serum and 50 μ M 2-mercaptoethanol (19-21). NIH 3T3, RS42, and the stromal

cell line S17 (22) were maintained in Dulbecco's modified Eagle's medium plus 10% fetal bovine serum. All cell lines were grown to a density of 10^6 cells per ml prior to isolation of RNA and protein.

RESULTS

Nucleotide Sequence of α_{14} and α_{15} . α_{14} and α_{15} were identified as unique α subunits based on the DNA and corresponding amino acid sequence of the cloned PCR fragments that were amplified from mouse spermatid cDNA between the degenerate oligonucleotide primers oMP19, -20, and -21 (Fig. 1; ref. 5). PCR fragments of α_{14} and α_{15} were subsequently used to screen lung and spleen cDNA libraries, respectively. The cDNA sequence of α_{14} (Fig. 1A) was obtained from three independent and overlapping clones. The sequence of α_{15} (Fig. 1B) was obtained from two overlapping cDNA clones that were isolated from independent libraries and several independently derived PCR products. The sequence of the two cDNA clones was identical in the overlapping region, except for a 162-nt insert in clone 781-1 at nt 745 that was produced by alternative splicing (data not

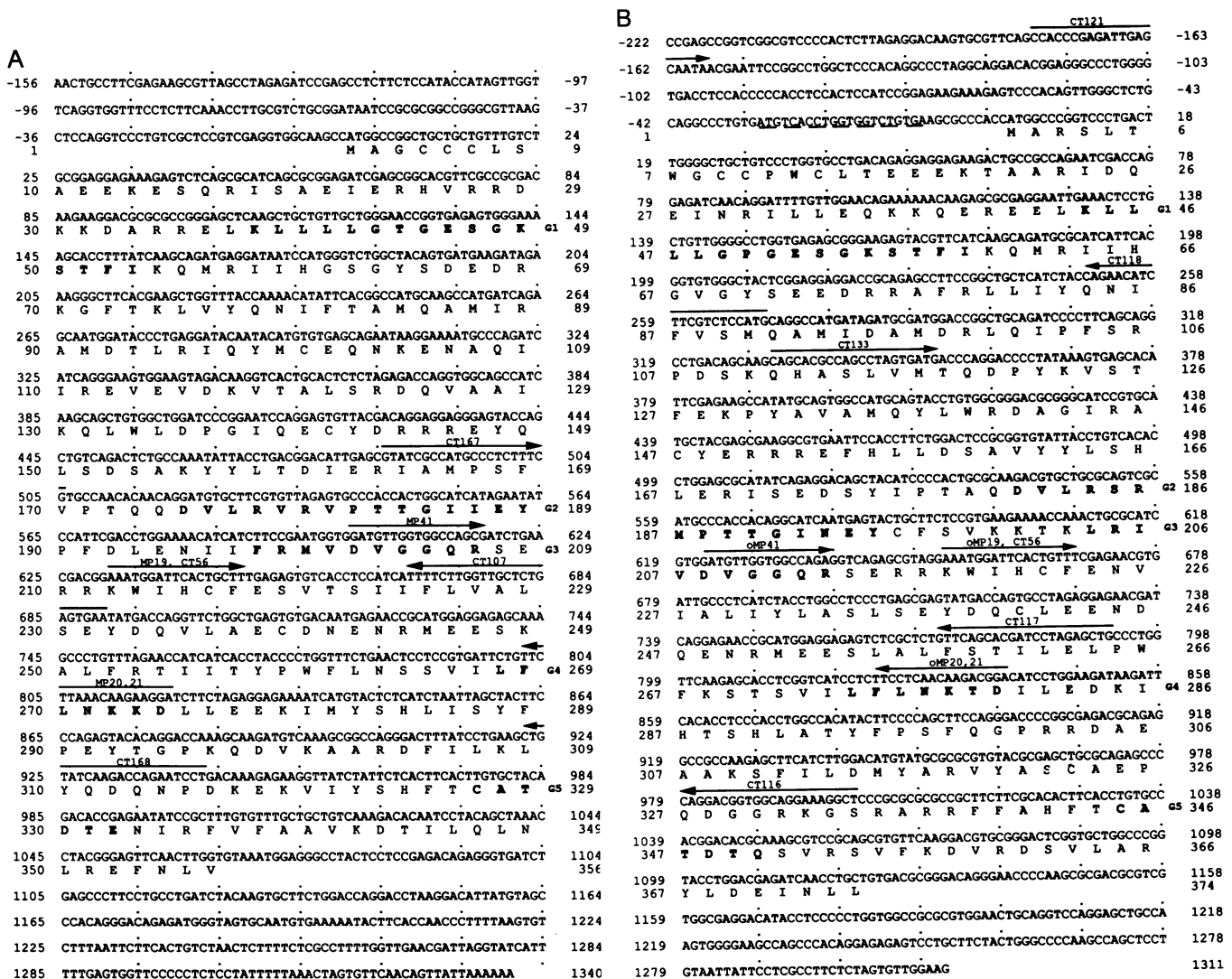


Fig. 1. cDNA sequence of α_{14} and α_{15} . The sequence of α_{14} (A) was obtained from three cDNA clones, the following nucleotides inclusive: clone 889-1 (876 to 1340); 889-2 (-156 to 1340); 889-5 (-156 to 496). The sequence of α_{15} (B) was isolated from two independent cDNA clones, the following nucleotides inclusive: 763-5 [563 to the poly(A) tail]; 781-1 (-222 to 1311). Oligonucleotides that were used for cloning and expression analysis are located below the arrows; left-pointing arrowheads indicate antisense oligonucleotides. The amino acids symbolized in boldface type are constituents of the conserved motifs that contribute to guanine nucleotide-binding and the conformational switch hinge, identified in the right margin as G1 through G5 (23, 24).

shown). This insert was not observed in the original PCR clones of $G\alpha 15$ obtained by PCR amplification between the degenerate oligonucleotides oMP19 and oMP21, nor was it observed in 15 independent PCR products that were amplified between CT133 and CT117 from cDNA of spleen and a variety of mouse hematopoietic cell lines. The sequence flanking the translation initiation site was also corroborated by directly sequencing the DNA products PCR-amplified from spleen cDNA in three independent reactions between the oligonucleotides CT121 and CT118 (Fig. 1B).

$G\alpha 14$ and $G\alpha 15$ encode proteins of 360 and 374 amino acids, respectively, that are predicted to be α subunits of heterotrimeric G proteins by virtue of high sequence identity to the conserved amino acids in other $G\alpha$ subunits. $G\alpha 14$ and $G\alpha 15$ are 81% and 58% identical to $G\alpha_q$, respectively, but only about 40% identical to all other $G\alpha$ subunits outside of the G_q class. We presume that translation of $G\alpha 14$ and $G\alpha 15$ initiates where indicated because these are the first methionine codons that are encountered in the coding sequence and they reside within the consensus sequence (25) just upstream of a region that is conserved among all $G\alpha$ subunits. $G\alpha 15$ contains a short open reading frame upstream of the initiation codon; this feature is shared only with $G\alpha 12$ (8) and $G\alpha 16$ (18) among the other α subunits and may provide a mechanism for translational regulation of these α subunits in some cell types.

PTX can block signal transduction generated from stimulated receptors by ADP-ribosylation of the GDP-bound form of certain α subunits. Members of the G_i class of α subunits that are sensitive to PTX each have a cysteine residue 4 amino acids from the carboxyl terminus. In contrast, α subunits that do not have cysteine at this position, including $G\alpha_s$, $G\alpha_{olf}$, $G\alpha_z$, and $G\alpha_q$, are not substrates for PTX (26, 27). Likewise, $G\alpha 14$ and $G\alpha 15$ lack a cysteine residue at this position, and consistent with this observation, protein extracts containing bacterially expressed $G\alpha 15$ are not ADP-ribosylated by PTX (V.Z.S., unpublished data). These α subunits are expected to mediate signal-transduction pathways that are refractory to PTX.

Tissue Distribution of α -Subunit mRNA from the G_q Class. The expression of $G\alpha 14$ and $G\alpha 15$ mRNA was assayed and compared with that of $G\alpha 11$ and $G\alpha_q$ mRNA in a variety of mouse tissues. The patterns of $G\alpha 14$ and $G\alpha 15$ expression were unique among the known mammalian α subunits. Tissue distribution was assayed by PCR amplification of randomly primed cDNA with oligonucleotides specific for each α subunit, and Southern blots of the PCR products were hybridized with oligonucleotide probes specific to $G\alpha_q$, $G\alpha 11$, $G\alpha 14$, or $G\alpha 15$ (Fig. 2). Coamplification with oligonucleotide primers specific to β -actin demonstrated that each sample contained comparable amounts of cDNA template. The patterns of $G\alpha_q$ and $G\alpha 11$ expression agreed with previous PCR and Northern analysis (7) and served as internal controls. Whereas $G\alpha 11$ was ubiquitous and $G\alpha_q$ was widely expressed, $G\alpha 14$ and $G\alpha 15$ were restricted in their tissue distribution.

The pattern of $G\alpha 15$ expression was of particular interest because it was apparently restricted to certain tissues, such as spleen, thymus, lung, and bone marrow (Fig. 2), that are rich in hematopoietic cell types. Macrophages are relatively abundant in the adult mouse lung, but spleen harbors hematopoietic cells of the myeloid, B, and T lymphoid lineages. $G\alpha 15$ mRNA was expressed in all seven samples that were assayed from normal mouse spleen and in the spleen of nude mice, which are deficient in T-cell production. Expression in thymus was lower than in spleen; $G\alpha 15$ may be relatively less abundant in T lymphocytes, developmentally restricted within this lineage, or predominantly expressed in other thymic cell types. $G\alpha 15$ mRNA was relatively abundant in embryonic liver (day 14) but undetectable in adult liver, suggesting that $G\alpha 15$ is also expressed early in hematopoietic

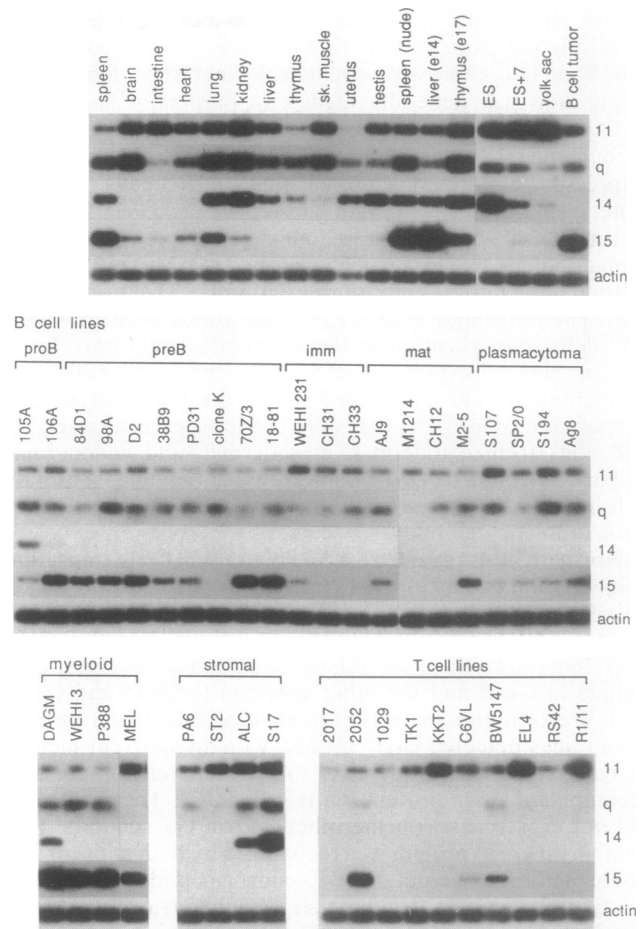


Fig. 2. PCR analysis of RNA expression in mouse tissues and cell lines. Randomly primed cDNA was amplified by PCR using oligonucleotide primers specific for each $G\alpha$ subunit in separate reactions. Coamplification with β -actin primers served as an internal control. The amplified products were blotted onto nylon filters and hybridized with a radiolabeled oligonucleotide specific to each gene. sk., Skeletal; e14 and e17, embryonic days 14 and 17; ES, embryonic stem cells; ES+7, embryonic stem cells after 7 days of differentiation; imm, immature; mat, mature.

development. $G\alpha 15$ was weakly expressed in yolk sac of day 8 embryos and in both proliferating embryonic stem (ES) cells and ES cells that had been differentiated to form blood islands in culture. $G\alpha 14$ mRNA was most abundant in spleen, lung, kidney, and testis and, interestingly, in ES cells but not in differentiated ES cells or yolk sac (Fig. 2). These same patterns of mRNA accumulation among the G_q class of α subunits were observed in separate PCR analysis using the degenerate oligonucleotide oMP19 or oMP41 paired with oMP20 or oMP21 (data not shown).

Expression of the G_q Class in Cultured Hematopoietic Cells. We analyzed RNA from cloned hematopoietic cell lines to determine the restrictions in hematopoietic expression among the α subunits of the G_q class. The PCR analysis was performed as described above using the two sets of degenerate oligonucleotides and, in separate reactions, each set of the specific oligonucleotides (Fig. 2). The analysis included RNA isolated from cell lines that were arrested at various maturational stages of B- and T-lymphocyte differentiation, cells from diverse myeloid lineages, four bone marrow adherent (stromal) cell lines, and many other nonhematopoietic cell types.

$G\alpha 11$ was expressed in all murine cell lines assayed. Hematopoietic expression of $G\alpha_q$ and $G\alpha 15$ was nearly parallel; both were expressed during all B-cell maturational

stages shown and in various myeloid cell lines but were generally not expressed in T cells. However, the pattern of $G\alpha_{15}$ and $G\alpha_q$ expression could be distinguished; first, $G\alpha_{15}$ expression was generally highest in progenitor and pre-B cells whereas $G\alpha_q$ expression was essentially constant throughout B-cell maturation, and second, $G\alpha_q$ was not expressed in murine erythroleukemia (MEL) cells. Of final note, each of the stromal cell lines expressed $G\alpha_{11}$ and $G\alpha_q$ but not $G\alpha_{15}$. $G\alpha_{14}$ was predominantly expressed early in hematopoietic lineages, such as in the DAGM (DA3.15; ref. 28) and progenitor B-cell cultures (29) and in S17 stromal cells (22). The progenitor B cells 105-A and 106-A were grown on S17 cells; hybridization in these samples could have been due, in part, to expression of $G\alpha_{14}$ in the feeder cells, but contamination did not exceed 5% of the nonadherent cell preparations. $G\alpha_{11}$ and $G\alpha_q$ were coexpressed in a variety of cell lines from ectodermal, mesodermal, and endodermal origins. In contrast, $G\alpha_{14}$ was expressed only in a subset of cell lines and $G\alpha_{15}$ expression was most significant within hematopoietic cell lines.

Northern blots revealed a 4.5-kilobase (kb) $G\alpha_{14}$ transcript (Fig. 3A) and two major $G\alpha_{15}$ transcripts of 2.4 kb and 2.2 kb (Fig. 3B). Northern blots also detected $G\alpha_{15}$ mRNA in 70Z/3 but not in NIH 3T3 or RS42 cell lines (data not shown). Rabbit polyclonal antisera were raised against two peptides, one unique to $G\alpha_{15}$ and the other common to $G\alpha_{15}$ and $G\alpha_{16}$ (18). Western blot analysis of membrane fractions from two B-cell lines detected $G\alpha_{15}$ protein with an apparent molecular mass of 42 kDa, identical to the bacterially expressed recombinant $G\alpha_{15}$ protein (r $G\alpha_{15}$; Fig. 3C). These antisera did not crossreact with the other known $G\alpha$ subunits. Recombinant $G\alpha_{15}$ protein was recognized by both antisera but not by a third antiserum, raised against a peptide sequence in the G1 box common to almost all $G\alpha$ subunits outside of the G_q class; this antiserum also failed to recognize $G\alpha_q$ (27).

The cell lines used to assay $G\alpha_{14}$ and $G\alpha_{15}$ expression grow indefinitely in culture and it is possible that expression of $G\alpha$ genes in a cell line is essential for maintenance of proliferation. Discrete amino acid substitutions at Arg¹⁸⁹ and Gln²²⁷ in $G\alpha_s$ (and the corresponding residues in $G\alpha_i$), which maintain the GTP-bound active state of these α subunits, have been correlated with hypertrophy and oncogenesis in certain tissues (30, 31). We examined the cDNA sequence of $G\alpha_{14}$ and $G\alpha_{15}$ in 11 and 15 of the highest expressing cell lines, respectively, to determine whether analogous mutations in $G\alpha_{14}$ and $G\alpha_{15}$ were correlated with cell proliferation. Direct DNA sequencing of the PCR products that were amplified between specific primers for $G\alpha_{14}$ (CT167 and CT168, Fig. 1A) or $G\alpha_{15}$ (CT133 and CT117, Fig. 1B) revealed that each cell line assayed had the same amino acid sequence as expressed in normal mouse tissues. Silent sub-

stitutions were found only in the first nucleotide of the Arg¹⁸⁰ codon in $G\alpha_{14}$ in two of the cell lines assayed.

DISCUSSION

The G-protein α subunits that are expressed in mammals can be grouped into four classes based on comparison of the primary structure of 16 distinct sequences (3). The α subunits share considerable amino acid identity within the conserved motifs that contribute to the GTP-binding pocket and the conformational switch domain. The four classes, termed G_s , G_i , G_{12} , and G_q , are primarily distinguished by their amino acid sequence outside of these regions. The α subunits reported here, $G\alpha_{14}$ and $G\alpha_{15}$, are most closely related to members of the G_q class.

Sequence comparisons suggest that $G\alpha_{14}$ may be more similar biochemically to the other members of the G_q class than is $G\alpha_{15}$. For example, the sequence of $G\alpha_{14}$ is identical to those of $G\alpha_q$ and $G\alpha_{11}$ within the GTP-binding domains and the conformational switch region, labeled G1 to G5 in Fig. 1, whereas $G\alpha_{15}$ has a number of amino acid substitutions. Sequence differences in these domains may influence the relative rates of GDP/GTP exchange or GTP hydrolysis (26). The $G\alpha_{14}$ and $G\alpha_{15}$ proteins are predicted to be similar in at least two important aspects held commonly with other members of the G_q class. The amino terminus of both proteins lacks the consensus site for myristoylation (32) that facilitates this posttranslational modification of several α subunits in the G_i class (33). Additionally, $G\alpha_{14}$ and $G\alpha_{15}$ lack the cysteine residue four amino acids from the carboxyl terminus that is ADP-ribosylated in the α subunits of the G_i class (1) and thus are predicted to be insensitive to PTX.

G proteins that are refractory to PTX have been shown to regulate signaling pathways in a variety of mammalian tissues and cell types (15). Therefore, we determined the mRNA expression pattern of the α subunits in the G_q class in various mouse tissues. $G\alpha_{11}$ and $G\alpha_q$ were expressed ubiquitously (7); in comparison, $G\alpha_{14}$ was expressed in a few tissues and $G\alpha_{15}$ was predominantly expressed in tissues that are rich sources of hematopoietic cells (Fig. 2). To further characterize expression of the G_q class within the hematopoietic compartment, we assayed expression of the four α subunits isolated from mouse in a variety of murine hematopoietic and stromal cell lines, in addition to cell lines derived from nonhematopoietic tissues. $G\alpha_{11}$ mRNA was expressed in every cell line tested. In contrast, $G\alpha_{14}$ mRNA exhibited the most restricted pattern within the hematopoietic compartment; it was expressed only in certain bone marrow adherent (stromal) cell lines, probably in progenitor B cells, and in some immature myeloid cell lines. $G\alpha_q$ was expressed in all nonhematopoietic tissues and cell lines that were assayed; it was also expressed in B cells and

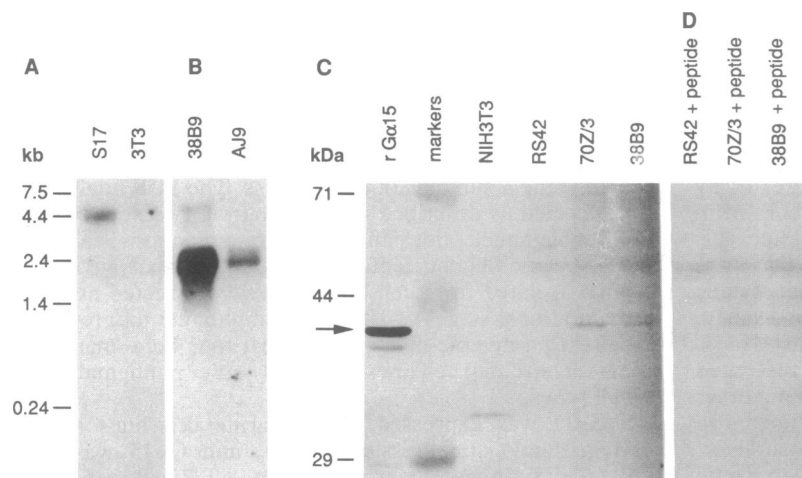


FIG. 3. Northern and Western analysis of $G\alpha_{14}$ and $G\alpha_{15}$. (A and B) $G\alpha_{14}$ (A) and $G\alpha_{15}$ (B) transcripts were detected on Northern blots containing 5 μ g of poly(A)⁺ RNA isolated from the indicated cell lines. (C) $G\alpha_{15}$ protein was detected in the membrane protein fractions (50 μ g) of two B-cell lines by Western blot analysis using affinity-purified polyclonal antiserum raised against a peptide sequence unique to the carboxyl terminus of $G\alpha_{15}$ in mice. (D) Detection of $G\alpha_{15}$ protein was blocked by coinubation of antisera with the $G\alpha_{15}$ peptide.

most myeloid cell lines but not in most T-cell lines. *Ga15* mRNA was expressed in all myeloid lineages that were analyzed and in B-cell lines that were arrested at various developmental stages from progenitor to mature cell types. *Ga15* was not expressed in most T-cell lines or in any of the stromal cell lines; it was very weakly expressed in NIH 3T3 and P19 cells and undetectable in other nonhematopoietic cell lines. Expression of *Ga_q* and *Ga15* in a few of the murine T-cell lines may reflect the previously observed expression of characteristically B-cell-restricted genes in immature T-cell tumors (34, 35). The general pattern of expression among murine T-cell lines suggests that *Ga11* is the predominant α subunit of the *G_q* class in T lymphocytes.

The expression pattern of *Ga15* is similar to that of human *Ga16*. Both genes are expressed predominately in hematopoietic lineages, although *Ga16* appears to be more prevalent in T-cell lines (18). *Ga15* and *Ga16* are probably the mouse and human homologues of a specific α subunit. Southern blots of mouse or human genomic DNA digested with several different restriction enzymes showed the same hybridization patterns when probed with either *Ga15* or *Ga16*, and chromosomal mapping data demonstrated that *Ga15* and *Ga16* cosegregated with the same two flanking loci in mouse and humans (T.M.W., N. Jenkins, N. Copeland, and A. Olsen, unpublished observations). These results were unexpected because *Ga15* and *Ga16* exhibit only 85% amino acid identity whereas the mammalian homologues of other α subunits are >95% identical.

Nine distinct PLC proteins have been identified (36) and, recently, *Ga_q* was found to mediate PTX-insensitive stimulation of PLC β 1 but not PLC γ 1 or δ 1 (14). Although the precise effectors in the signal-transduction cascades mediated by *Ga14* and *Ga15* are unknown, their similarity to *Ga_q* suggests that they may also activate the PLC β isotypes. Indeed, transient transfection assays suggest that all members of the *G_q* class can activate PLC β 1 (C. H. Lee, D. Wu, and M.I.S., unpublished observations). It is not clear from this result why some cell types express all four members of the *G_q* class if the primary role of these α subunits is to activate PLC. Perhaps they differentially activate the PLC β isotypes *in vivo*. Other effector interactions are possible and not necessarily mutually exclusive, including regulation of various ion channels. The α subunits in the *G_q* class could also be functionally distinguished by their receptor interactions. With the exception of *Ga11*, expression of these α subunits within the hematopoietic compartment is restricted to certain lineages or developmental stages. An attractive model is that these α subunits are components of specific signaling pathways that mediate hematopoietic and stromal cell interactions, thus contributing to the regulation of hematopoiesis.

Note Added in Proof. The mouse *Ga14* cDNA is homologous to the bovine *G_{L1a}* cDNA reported by Nakamura *et al.* (37).

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